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Author Affiliation:

Department of Anatomy, Faculty of Basic Medical Science,
University of Uyo, Main Campus, Ikpa Road Uyo, Akwa Ibom
State, Nigeria

*Corresponding Author

Department of Anatomy, Faculty of Basic Medical Science,
University of Uyo, Main Campus, Ikpa Road Uyo, Akwa Ibom
State,
Nigeria
Email: unwanamishaël@gmail.com

Contact List

Unwana E Mishaël	unwanamishaël@gmail.com
Idorenyin Umoh	Idorenyinumoh@uniuyo.edu.ng
Innocent A Edagha	innocentedagha@uniuyo.edu.ng
Eno-obong I Bassey	enoobongbassey@uniuyo.edu.ng

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Effect of Alcohol toxicity on estrous cycle, and in the uterus of Wistar rats

Unwana E Mishaël*, Idorenyin Umoh, Innocent A Edagha,
Eno-obong I Bassey

ABSTRACT

In investigating the effect of Alcohol on the estrous cycle and uterine microstructure, hormonal and anti-oxidant concentration, twenty; adult female Wistar rats weighing 95 to 155 g were used. The animals were grouped into four groups of five rats each; group 1 is the control; while the animals in group 2- 4 received 0.13 mL/kg, 0.67 mL/kg and 1.34 mL/kg of Alcohol. The administration was given with an orogastric tube orally for 28 days, weighed and sacrifice on the 29th day, using ketamine injection intraperitoneally. Blood, Uterus were harvested for hormonal and biochemical assays. The Uterus were processed using Hematoxylin and Eosin staining techniques and Masson trichrome stain for cyto-architectural assessment. The estrous cycle for the animals in group 1 had a regular cycle, Alcohol groups had irregular cycle, an increase in body weight of the animals in all the groups were recorded. Sections of Tissue showed hypertrophy of uterine glands in the high dose of alcohol group and Thickening; of epithelial lining for animals in group three. Alcohol groups had decreased staining intensity for the collagen fibers in the uterus. Catalase, Sodium dismutase activities decreased in group three and group four. Hormonal; concentrations decreased in alcohol groups with a statistical significance value of $p < 0.05$. Alcohol; can cause irregular estrous cycle, enlargement of the endometrial gland with symptoms such as menorrhagia, dysmenorrhea, anaemia and can also affects implantation of the foetus.

Keywords: Estrous cycle, Wistar rats, Myometrium, Endometrium

1. INTRODUCTION

Alcohol drinking has become increasingly popular, which has highlighted the prevalence of several types of fatal illnesses. This includes the usual menstrual disorder, hormonal adjustment, as well as long-term impacts that might become apparent as one ages. The majority of women nowadays ritualistically consume highly concentrated alcoholic beverages regularly, assuming an addicted trajectory, without giving the negative implications this lifestyle poses on the female reproductive systems enough thought (Ashley et al., 2019). This trend may lead to an acute dependence on Alcohol, leading to alcoholism (Brust, 2005; Boden and Fergusson, 2011). Alcohol; can cause inflammation. Alcohol; is a toxic

substance which can disrupt bacterial balance in the body, making the immune system weak (LeClercq et al., 2017).

This allows the body to be susceptible to a myriad of illnesses including, reproductive anomalies in women (Kristin and Brooke, 2017). Alcohol harms the reproductive system; studies have shown that drinking can alter ovulation, produce irregular menstrual cycles, and result in ovarian cysts. Alcohol; interferes with healthy implantation in the uterus, increasing the likelihood of preterm birth. Alcohol disrupts the ovulation cycle and hormone synthesis (Angelis et al., 2020). Menstrual problems, amenorrhea, anovulation, luteal phase dysfunction (issues with the endometrial lining), early menopause, heavy menstrual flow, and dysmenorrhea are all symptomatic effect of alcohol consumption (LeClercq et al., 2017; Angelis et al., 2020). The general objective of this research work was to study the effects of alcohol toxicity.

The specific objectives were:

Effect of Alcohol on the estrous cycle and the cytoarchitecture of the uterus

Effect of Alcohol on hormonal and biochemical activities using blood

2. METHODOLOGY

Materials

A brand of Alcohol; Lax Vegas Authentic brandy with the number, NPN: 01-5668, Source and purchase from Mainland supermarket in Uyo.

Source and Maintenance of Animals

Twenty adults female Wistar rats weighing 95 – 155 g were used for the experiments. They animals were grouped into four groups of five rats each. The rats were purchase and house in the Pharmacological house of the Faculty of Pharmacy, University of Uyo, in clean cells with bedding. Wistar rats were given rat pellet diet, and allow access to water ad libitum; animals are also allowed to acclimatise for two weeks before the commencement of the research and exposed to twelve hours light and twelve hours dark cycle at room temperature of 27 °C-30 °C.

Test Solution and Administration

Lorke's method is use for the determination of the median lethal dose for substances. LD 50; of the Alcohol is 13.42 mL. Alcohol; groups receive 1% of 13.42 (1.34 mL/kg), 5% of 13.42 (0.67 mL/kg) and 10% of 13.42 (1.34 mL/kg) of Alcohol. The administrations were given orally, daily, for 28 days.

Estrous Cycle Study

Estrous cycle study is done by the quantifying of cells found in vagina smears collected from Wistar rats. The rat vagina orifice is washed with distilled water; a few drops of normal saline is drawn using a pipette and inserted about 2- 3 mm into the vagina orifice. The pipette will be removed with the aspirated fluid, dropped in a clean slide, allowed to stay. A, drop of Eosin stain is added to the slide, and viewed on a microscope.

Termination of the Experiment

After the last day of administration, on the 29th day, the animals were weighed, sacrificed, from all the groups. The rats were anesthetized with Ketamin injection intraperitoneally, Blood specimen was collected from the right ventricle of the heart, into sample bottles for hormonal and anti-oxidant assay. The uteri were harvested from all the groups, and grossly examined for the presence of anatomical alterations or artifacts; the tissues were then fixed in 10% buffered formalin to prevent autolysis, Processed for Histological Studies.

Ethical Issues

Experimental procedures involving the animals are in accordance with International Guidelines for the care and use of laboratory animals and the Faculty of Basic Medical Sciences Research and Ethical Committee University of Uyo.

3. RESULTS

The result for the estrous cycle is presented in a clustered bar chart, as shown in (Figure 1).

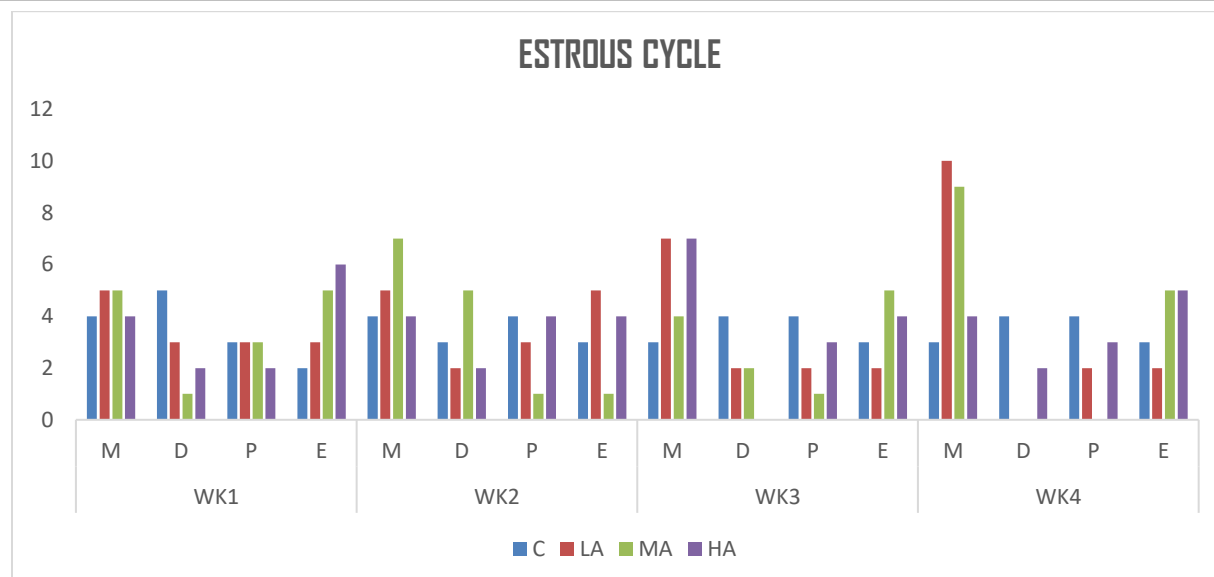


Figure 1 C: Control; LA: Low dose of Alcohol; MA: Medium dose of Alcohol; HA: High dose of Alcohol; E: Estrous; D: Diestrus; P: Proestrus M: Metestrus

Result from histological sections of animals in control (distill water), Group 2 (0.13 mL) of Alcohol, Group 3 (0.67 mL) of Alcohol and Group 4 (1.34 mL) of alcohol. Stain in Haematoxylin & Eosin (H & E) X 100 are presented in Figure 2, while sections stain with Masson Trichrome stain (MT) X 100 are represented in (Figure 3).

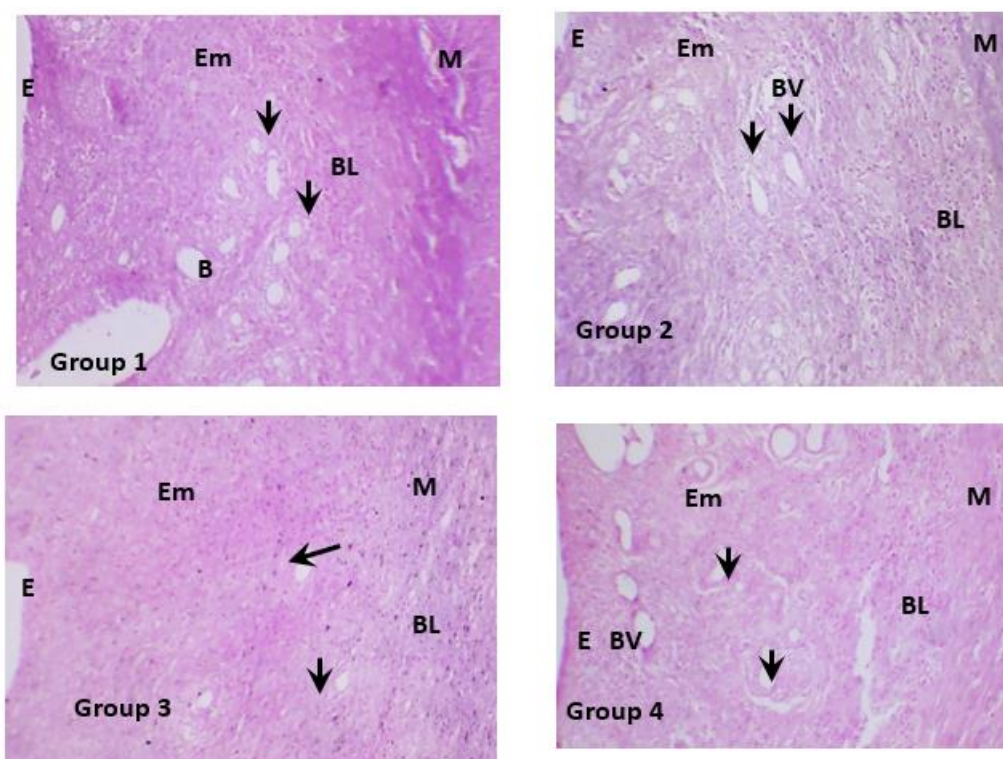


Figure 2 Photomicrograph of the longitudinal section of the uterus of rats in normal control (Group 1), shows normal cytoarchitecture of the uterus with the myometrium (M) and endometrium (E). The endometrium appearing normal with numerous glands (arrow), blood vessels (BV), and a high density of basal cells (BL). Group; 2 shows thickening of the epithelium, with slightly thinner flattened cells. Group; 3 shows more minor thinned wall glands. Group; 4 shows hypertrophy of the glands, H & E X 100.

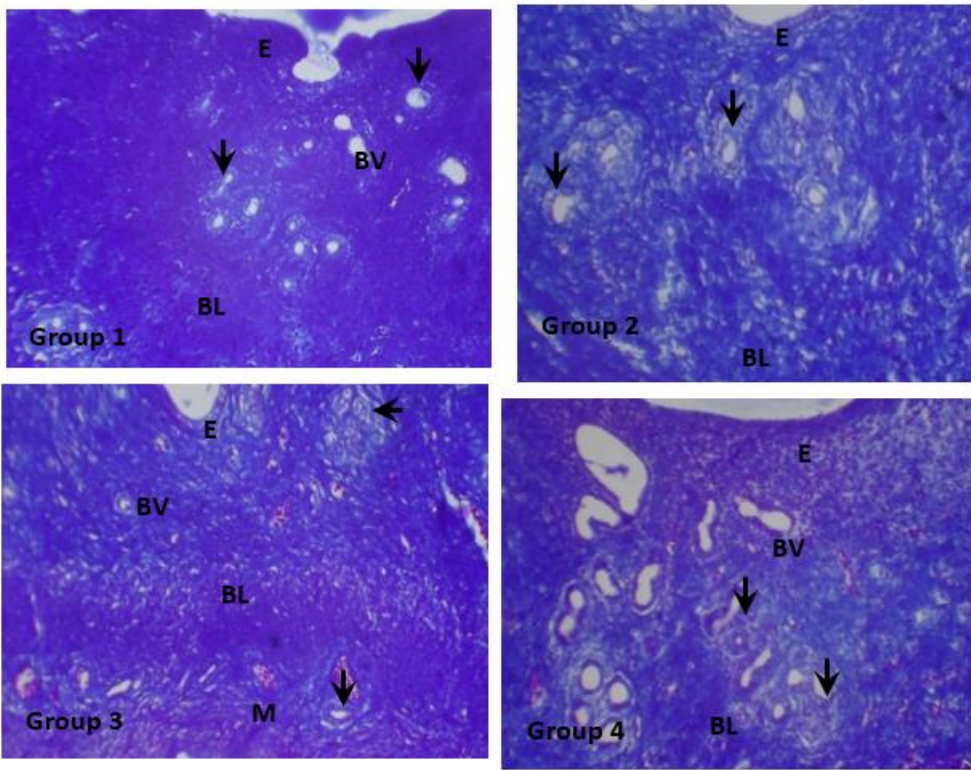


Figure 3 Photomicrograph of the longitudinal section of the uterus of rats in Group 1 normal control showing well-stained collagen (C) in the uterine walls. The glands and muscles were less stained. Group; 2 shows less stained collagen. Group; 3 shows less stained collagen. Group; 4 showed hypertrophy of the gland with less stained collagen, MT x 100

Table 1 Result of biochemical (CAT, SOD) and Hormonal (Estradiol, FSH, LH) Assay

	Group 1	Group 2	Group 3	Group 4
CAT	79±0.39	82±2.3	74±2.1	71±3.3
SOD	28±0.74	29±0.49	25±0.99	24±0
E2	18±2.2	20±2.4	20±6.4	29±3.2
FSH	2.2±0.2	2±0.055	1.9±0.12	2.2±0.15
LH	2.2±0.21	2.1±0.18	1.90±0.11	2.4±0.26

Data is express as mean ± SEM at P < 0.05.
CAT; Catalase activity, SOD; Sodium dismutase, E2; Estradiol, FSH; Follicle stimulating hormone, LH; Luteinizing hormone

4. DISCUSSION

The estrous cycle is the rhythmic reproductive cycle of different day’s duration occurring in a sexually matured female animal (Ajayi and Aklhigbe, 2020). The average length of the estrous cycle in rodent is 4-5 days; they phases are identify as proestrus characterized by the presence of nucleated epithelial cells. Estrus; is characterized by clusters of cornified cells that were primarily non-nucleated. Metestrus; is characterized by a mixture of numerous leukocytes and a few cornified cells. Diestrus; is suggested by only leukocytes. For; the 28-day experiment taking the average that the animal estrous cycle is four or five days, cycle phase was to occur six-eight times throughout the experiment for the animal to have a regular cycle. All; the animals in the control group had a regular cycle. Group; 2 animals cycles phase occur as follows; high metestrus, with metestrus occurring seventeen times, low diestrus phase occurring three times, low proestrus phase occurring two times, standard estrus phase occurring six times.

The animals in group 2 experience an irregular cycle. Group; 3 animals cycles phase were irregular, occurring as follows high metestrus phase occurring fourteen times, normal diestrus phase occurring six times, low proestrus phase occurring two times, typical estrus phase occurring six times. Group; 4 animals cycles phase were irregular, occurring as follows, high metestrus phase occurring nine times, low diestrus phase occurring three times, regular proestrus phase occurring seven times, high estrus phase

occurring nine times. The normal histo-cytology of the uterus is of three layers, the outer perimetrium, middle myometrium, an inner endometrium. The perimetrium is line by the serosa. The endometrium is line by simple columnar epithelium that extends inward to form numerous uterine glands (Mescher, 2010). At a non-proliferative stage, it contains mainly the basalis layer with the basal remnants of the uterine glands.

The myometrium consists of a thick, smooth muscle bundles seen in the cross, the oblique, and in the longitudinal sections. A thin strand, of interstitial connective tissue with numerous blood vessels separates the smooth muscles. The lamina propria of the endometrium contains collagen fibers with abundant fibroblast and ground substance (Mescher, 2010). Sections of the animals in the control group contain collagen, well-stained all through the uterine walls for the Masson Trichrome stain. In; Haematoxylin and Eosin stain, the uterus endometrium is normal with numerous glands and blood vessels. Cells of animals in a low dose of alcohol (0.13 mL/kg) group contain less stain collagen on its uterine wall for MT stain, and thickened epithelium with numerous distributed glands. A; slightly flatten cells were observe for the H & E stain. The animals administer with 0.67 mL/kg of Alcohol had a less stain collagens for MT sections, while the H & E sections, showed few smaller-size thinned wall glands, and thinning of endometrium. Group; given 1.34 mL/kg of Alcohol; showed less stain collagen for MT, and enlarged glands, and cells of the basalis layer for its H & E sections.

A normal endometrium of the female in a non-proliferative stage is about 8 mm. Thickening; of the endometrium and hypertrophy of glands as observed in group 4, is an irregular condition, which may cause uncomfortable symptoms for women, including menorrhagia, anemia, and pains in the abdominal region. Thinning; of the endometrium or glands observed in group 3, can impact fertility in several ways. When; the endometrium becomes too thin; it will not be strong enough to anchor a fertilized egg, thereby affecting implantation. Effect; on anti-oxidant concentration; Superoxide dismutase (SOD) concentrations and Catalase activity are reduced for groups 3 and 4, but increased in group 2. Decrease; in anti-oxidant activities suggest that uterine tissues in the female reproductive system will not have protection against oxidative damage that may arise due to Alcohol administration. A; balance between reactive oxygen species (ROS) production, and elimination is essential for optimal functioning of the female reproductive system to ensure normal oocyte maturation, corpus luteum formation, ovulation, fertilization, and implantation (Zhang et al., 2016; Katz-Jafe et al., 2020).

The effect on FSH concentration was significantly decreased in group 2 but increased in group 3 and group 4. Estradiol; concentration was significantly increased for group 2, group 3, and group 4. LH; concentration increase at $p < 0.05$ for group 2, group 3, and group 4. Estrogen; and LH act in a paracrine manner to enhance FSH. FSH; stimulates the follicles on the ovary to grow and prepare the eggs for ovulation. As; the follicles increase, they begin to release estrogen and a low level of progesterone into the blood. Observations; and experimental studies have demonstrated that moderate to acute alcohol consumption affects the endocrine systems in women by either elevating or suppressing hormonal levels (Angelis et al., 2020; Das and Vasudevan, 2007).

5. CONCLUSION

Alcohol can cause irregular estrous cycle, hypertrophy of the endometrial gland of the uterus. This condition can result in uncomfortable symptoms for the women such as menorrhagia, dysmenorrhea, post- menopausal bleeding, and anemia. Alcohol; can impact implantation, if the endometrium becomes too thin, the uterus will not be able to anchor the fertilize egg.

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Author Contributions

The design of the study, research and manuscript writing is by Unwana Mishael.

The statistical analysis is by Idorenyin Umoh

The interpretation of the result and discussion is by Innocent Edagha

Proof reading is by Eno-obong Bassey

Ethical approval

The study was approved by the Faculty of Basic Medical Sciences Research and Ethical Committee (Ethical approval number: UU_FBMSREC_2023_001). The Animal ethical guidelines are followed in the study for experimentation.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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